EFFECTS OF AMMONIUM, NITRATE AND PHOSPHATE ON THE GROWTH OF *CYSTOSEIRA STRICTA* (PHAEOPHYTA, FUCALES) CUTTINGS IN CULTURE

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ABSTRACT -- The effect of different concentrations of ammonium, nitrate and phosphate on the growth of *Cystoseira stricta* cuttings were studied. Cuttings were cultivated under different concentrations of added nutrients (0 to 2.35 mM NO_3, 0 to 0.625 mM NH_4^+, 0 to 0.125 mM PO_4) and similar light, temperature and emersion - immersion conditions. Their growth is stimulated by nitrate supply whereas phosphate addition does not increase their development rate. Highest concentrations of tested enrichments result (1) in a growth rate lower than that of the control plant and (2) in the roting of the plants. Nature of the nutrient added also influenced morphological development and pigmentation of the cuttings.

RÉSUMÉ – Notre étude porte sur l'influence de différentes concentrations ▬ ammonium, nitrate, phosphate sur la croissance des boutures de *Cystoseira stricta*. Les teneurs ▬ ▬ différents sels nutritifs varient de ▮ à 2,35 mM pour NO_3^-, de 0 à 0,625 mM NH_4^+ et de 0 à 0,125 mM pour PO_4^{3-}. Un enrichissement en nitrate est nettement plus favorable à la croissance qu'un enrichissement en phosphate. L'aspect morphologique ■ la pigmentation sont également fonction de la nature de l'enrichissement. Des concentrations trop élevées en ces sels nutritifs induisent un taux de croissance inférieur à celui des boutures témoins et provoquent le pourrissement des boutures.

KEY WORDS : *Cystoseira*, culture, nitrogen, phosphate, enrichment.

INTRODUCTION

As with higher plants, the development of numerous algae is affected by nutrient supplementation of the culture medium. Nitrogen fertilizers are generally considered to stimulate plant growth and to intensify pigmentation. Such a phenomenon has been described for Rhodophyta e. g. for *Chondrus crispus* and *Palmaria palmata* (Neish *et al.*, 1977; Morgan *et al.*, 1980) and for Phaeophyta : *Laminaria* (Chapman *et al.*, 1978). Studies about phosphate requirements have shown that this compound is necessary to obtain suitable development of microscopic stages of brown algae : *Lessonia nigrescens* (Hoffman *et*

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al., 1984), *Laminaria saccharina* (Hsiao & Druehl, 1973). Chemical composition and regenerative properties of *Cystoseira stricta* lead us to study the influence of such nutrients on the growth, morphological differentiation and pigmentation of cuttings of this species.

MATERIAL AND METHODS

Numerous thalli of *Cystoseira stricta* Sauvageau (Phaeophyta, Fucales) were collected at Carro (Southeastern France near Marseille) in early December 1984, at the beginning of the growth period. One thousand six hundred young primary axes were cut. Cuttings were prepared as described in a preceding paper (Epiard-Lahaye *et al.*, 1987) : from each primary axis, apical and subapical segments were collected. All these cuttings were 1 cm long. Statistical minimum sample size was calculated from preliminary experimentation using the formula : $n = \frac{4\sigma^2}{d^2}$ were d is the maximal error, σ is the standard deviation and the confidence level is 95 % (Dagnelie, 1973). The result was that 36 cuttings were necessary to obtain a statistically significant sample.

From a larger collection of segments, 40 cuttings from each of two sorts of segments were selected at random. Two samples of apical segments and two samples of subapical segments were placed in each culture tank. At the beginning of the culture, plant tissue total weight, in each tank, varies from 6.5 to 7.0 g. The free-living system used for these cultures (Pellegrini & Lahaye, 1987) consists of eighteen modules arranged in a thermostatically controlled water. Each module is composed of (1) a superior culture tank (2) a central transfer tank and (3) a lower feed-pump. The feed pump draws water from the transfer tank to the culture one. The water reaches a siphon and then an overflow. When the pump stops, the siphon empties the culture tank. The siphon fails and the cycle starts again. The samples were exposed to an emergence-submergence cycle of 5 min/10 min. This cycle has been determined to be the best for the development of the cuttings (Epiard-Lahaye *et al.*, 1987).

Temperature of the room and of the culture medium was maintained at 16±1°C. The light over each section of 6 culture tanks was provided by four fluorescent lamps : 2 cool-white (Claude, 52 W) and 2 Gros-lux (Sylvania, 52 W). The quantum irradiance received by the cuttings was 100 $\mu Em^{-2}s^{-1}$. The culture medium was natural seawater from Carro, 25 liters in each culture tank, enriched or not with mineral salts. Salinity was regulated at 38 ‰. Daily water analysis (for NO_3, NO_2, NH_4 and PO_4) showed that the medium in each culture unit had to be changed weekly, except for NH_4NO_3 for which it had to be renewed every three days. The procedure used for analyses of N was colorimetry after Koroleff (1969). The measurement method for P was colorimetry after Murphy & Riley (1962). Aeration of the seawater was provided by a regulated bubbling of compressed air in each culture tank.

The investigation relates, at the same time, to the nature and to the concentration of the enrichment. The influence of five different salts was studied in

Number of the tank	Nature of the enrichment	Concentration of NO_3^- or PO_4^{3-} (mM) added.
1	KNO_3	0.69
2		0.99
3		1.98
4	$NaNO_3$	0.59
5		1.18
6		2.35
7	NH_4NO_3	0.13
8		0.25
9		0.63
10	KH_2PO_4	$3.7.10^{-3}$
11		$14.7.10^{-3}$
12		$73.5.10^{-3}$
13	NaH_2PO_4	$41.7.10^{-3}$
14		$83.3.10^{-3}$
15		$125.0.10^{-3}$
16	unenriched seawater	■ $P\text{-}PO_4$: $0.32.10^{-3}$ * $N\text{-}NO_3$: $0.25.10^{-3}$ * $N\text{-}NH_4$: $0.15.10^{-3}$

Table I : Nature and concentrations of the nutrients added to the culture medium.
* These concentrations represent an average value of our weekly measurements during the experiment.

the development regeneration and pigmentation of *Cystoseira stricta* cuttings. For each mineral salt, three concentrations were tested (Table I). The results were compared to those obtained in unenriched seawater. The averages of the naturally occurring concentrations in the Mediterranean Sea, at the collection station in winter (measurements in 1976, 1977, 1978) were :
$N\text{-}NO_3 = 3.0.10^{-3}$ mM and $P\text{-}PO_4 = 0.15.10^{-3}$ mM (Arfi, 1984).

Growth was measured by fresh weight determination of each sample (40 cuttings) at weekly intervals during a period of nine weeks. At the end of the experimentation, the plants were blotted, frozen and freeze-dried for further

analysis. Freeze-dried material was treated for spectrophotometric pigment analysis. Pigment extraction is carried out using 90 % acetone. Estimation of the pigment content is made according to the equations of Jeffrey & Humphrey (1975).

After each fresh weight measurement, the cumulative growth percentage of all the samples was calculated by using the formula :

$$P = \frac{(m - m_o)\,100}{m_o}$$

P : cumulative growth percentage
m : fresh weight at time t
mo : fresh weight at time 0.

Time 0 is the first day of the experiment, when the cuttings are placed in the culture tanks.

All data were statistically treated with linear regression method and the differences between the regression coefficients were tested by the Student's t-test ($p = 0.05$).

RESULTS

Although small differences exist between apical and subapical segments, the results obtained for these two sorts of cuttings compare well. Growth percentages values calculated for subapical segments are generally lower than those obtained for apical segments. This can be explained by the two weeks reaction time necessary for the edification of the neoformation buds.

Nitrate and ammonium enrichments.

The effect of nitrate fertilization on growth rate is striking, especially when the enrichment added to the medium is KNO_3 or $NaNO_3$. Concentrations listed are significantly higher than the naturally occurring concentrations in the sea.

Data presented in Table II and in figs 1 and 2 show that the highest growth rates (600 to 700 % at the end of the culture) appear with the smallest enrichment concentration tested : KNO_3 = 0.69 mM and $NaNO_3$ = 0.59 mM. Figs. 1 and 2 show that the maximum growth percentage is nearly reached by the control plants, as early as the sixth week, whereas cuttings cultivated in the lowest enriched media continue to grow up to the end of the experimentation. From these figures, it is important to note that addition of excess KNO_3 or $NaNO_3$ (1.98 or 2.35 mM) results in a significantly smaller enhancement of plant weight (173.6 and 342.8 % for apical segments; 228.1 and 256.6 % for subapical segments). Cuttings cultivated in such conditions are richly pigmented but they begin to rot during the last week of the experimentation. Growth rate is saturated at a NO_3 concentration between 1 and 2 mM.

Data obtained for NH_4NO_3 is different from that obtained for KNO_3 or $NaNO_3$. Daily water analysis show that there is a clear preference for ammonia

Nature of the salt	Concentration mM	Apical segments			Subapical segments		
		P ± SE	R	a	P ± SE	R	a
Unenriched seawater		328.1 ± 8.9	0.959	36,99	253,5 ± 9.1	0.974	27,94
KNO_3	0.69	706.9 ± 4,9	0.985	81,36	691.7 ± 124.4	0,982	81,27
	0.99	548.3 ± 38,9	0.994	66,12	396.8 ± 0	0.988	48,65
	1.98	'173,6 ± 25,5	0,972	20.08	'228,1 ± 30.0	0.989	27.46
$NaNO_3$	0.59	634.5 ± 36,5	0.984	77,82	487.5 ± 0	0,987	56,60
	1,18	466,5 ± 44,6	0.996	55,34	447.9 ± 10,3	0.991	52,43
	2,35	342,8 ± 10,1	0,997	39.29	256,6 ± 13.5	0.996	30,62
NH_4NO_3	0.13	423,1 ± 21,3	0,996	51,10	452,8 ± 2,0	0.993	56,71
	0,25	'437,1 ± 4,1	0.994	59,76	'367,6 ± 22,9	0.994	45,31
	0,63	*262,1 ± 2,4	0,998	39,26	*253.5 ± 6,4	0.988	39,55
KH_2PO_4	$3.7.10^{-3}$	** 96,5 ± 2.5	0.976	26,66	** 70,0 ± 2,3	0,979	19,34
	$14.7.10^{-3}$	215,4 ± 10.9	0.971	25,22	220,2 ± 7,8	0,975	27,67
	$73.5.10^{-3}$	*152,2 ± 13,3	0,963	20,14	*136,1 ± 2,0	0.979	18,96
NaH_2PO_4	$41.7.10^{-3}$	266,5 ± 3,5	0,963	30,28	255,0 ± 20,7	0,989	31,75
	$83.3.10^{-3}$	328,6 ± 20,2	0,962	37,30	257,5 ± 47,7	0,979	31,46
	$125.0.10^{-3}$	331,1 ± 10,7	0,969	38,07	245,3 ± 5,8	0,983	29,49

Table II : Final growth percentages of the cuttings grown at 3 concentrations of 5 different enrichments and grown in an unenriched seawater, and their corresponding linear regression coefficients (means ± 95 % confidence limits).

P : growth percentage at the end of the culture; SE : standard error; R : correlation coefficient; a : slope of the regression line; ' : only 8 weeks of culture; '' : only 7 weeks of culture; ''' : only 4 weeks of culture.

as a nitrogen source (Fig. 3). Therefore, this double nitrogen source does not seem to be the best for the growth of *Cystoseira stricta* cuttings. Such an enrichment (0.13 and 0.25 mM N-NH_4) results in an increasing of the weight only 1.3 times higher than that obtained in an unenriched seawater (Tableau II, Fig. 4). When N-NH_4 concentration equals 0.63 mM (total N = 1.26 mM), growth rate of the cuttings is similar to that of the control plants and significantly lower than those obtained in a 1.18 mM N-NO_3 (as $NaNO_3$) enriched media. Addition of excess nitrogen causes the decay of the plants.

Data presented in these figures establish that substantial growth occurs by addition of the smallest amounts of KNO_3 or $NaNO_3$ tested . These different growth rates are reflected in appreciable morphological variations (Figs. 5 and 6). Cuttings submitted to KNO_3 or $NaNO_3$ enrichment show appreciable morphological differences one another, they are richly pigmented, highly bran-

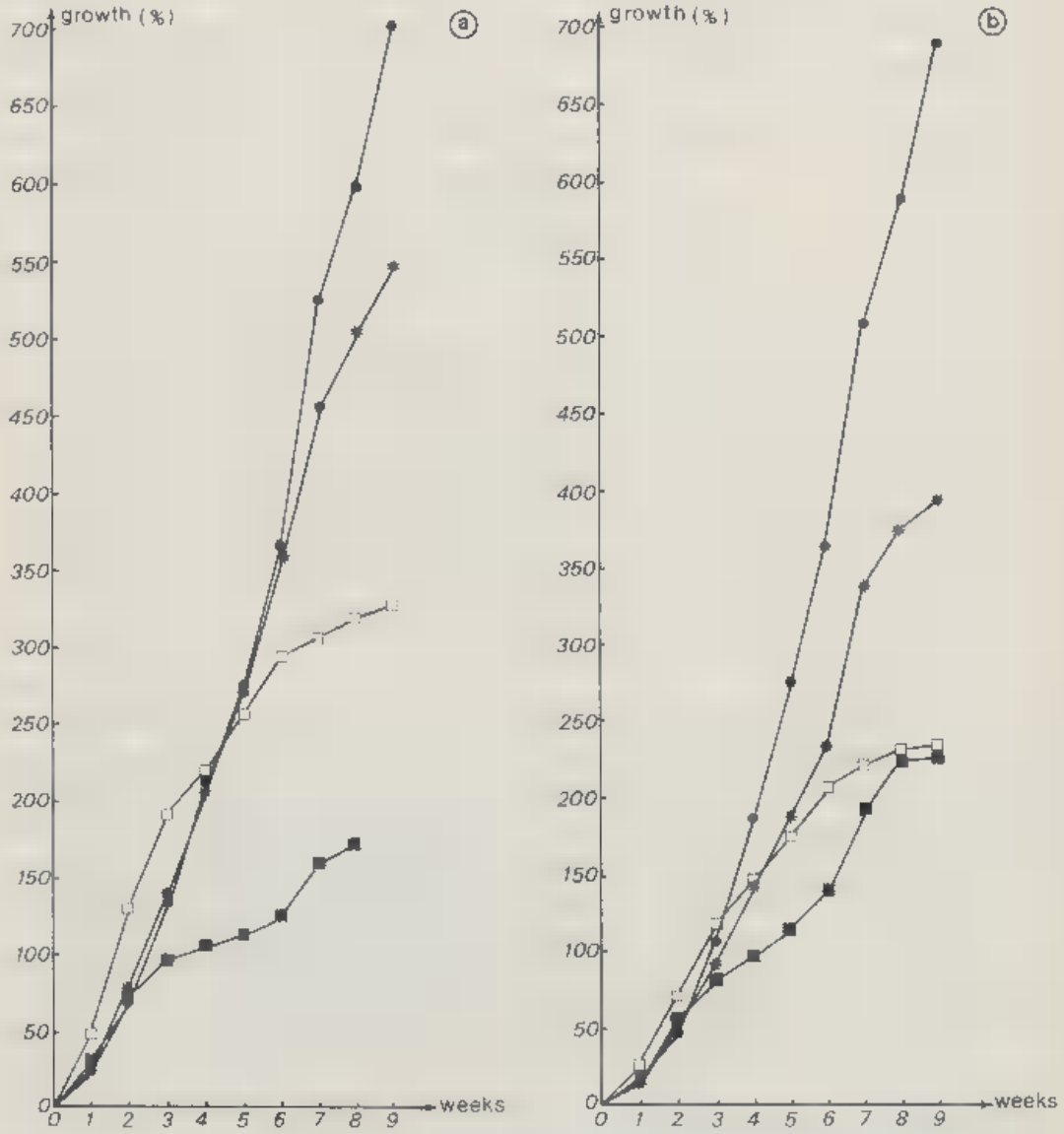

Fig. 1 — Cumulative growth of cuttings fertilized with nitrate (KNO_3) or not (controls). Results expressed in different percentages of the initial fresh weight.
a : apical segments, b : subapical segments.
■ : 0.69 mM N-NO_3, ★ : 0.99 mM N-NO_3, ■ : 1.98 mM N-NO_3, □ : control.

ched and develop numerous neoformation branches (Fig. 5b and 6b). Addition of NH_4NO_3 results in very long and delicate fragments (Figs. 5c and 6c). The tips of the new branches are poorly pigmented. Pigments analysis show that KNO_3 supplementation of the medium results in fragments as pigmented as material just collected. Total chlorophyll content decreases when fragments are sub-

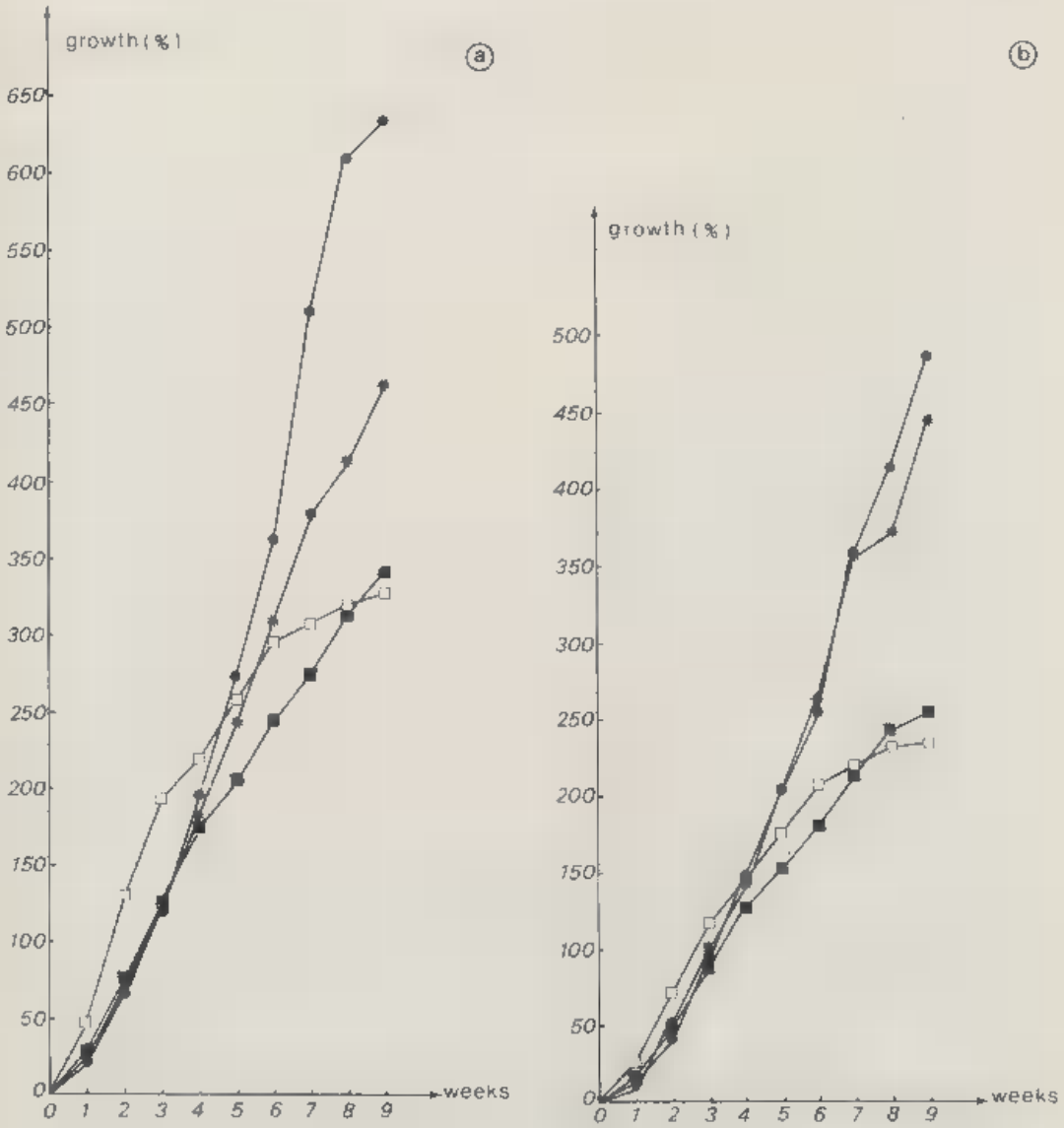

Fig. 2 — Cumulative growth of cuttings fertilized with nitrate ($NaNO_3$) or not (controls). Results expressed in different percentages of the initial fresh weight.
a : apical segments, b : subapical segments.
■ : 0.59 mM N-NO_3, ★ : 1.18 mM N-NO_3, ■ : 2.35 mM N-NO_3, □ : control.

mitted to a $NaNO_3$ enrichment. Addition of NH_4NO_3 results in less pigmented cuttings. Total chlorophylls and carotenoids contents are lower than in axes just collected. However, values obtained after N enrichment are higher than those obtained for the control cultivated in an unenriched seawater (Table III).

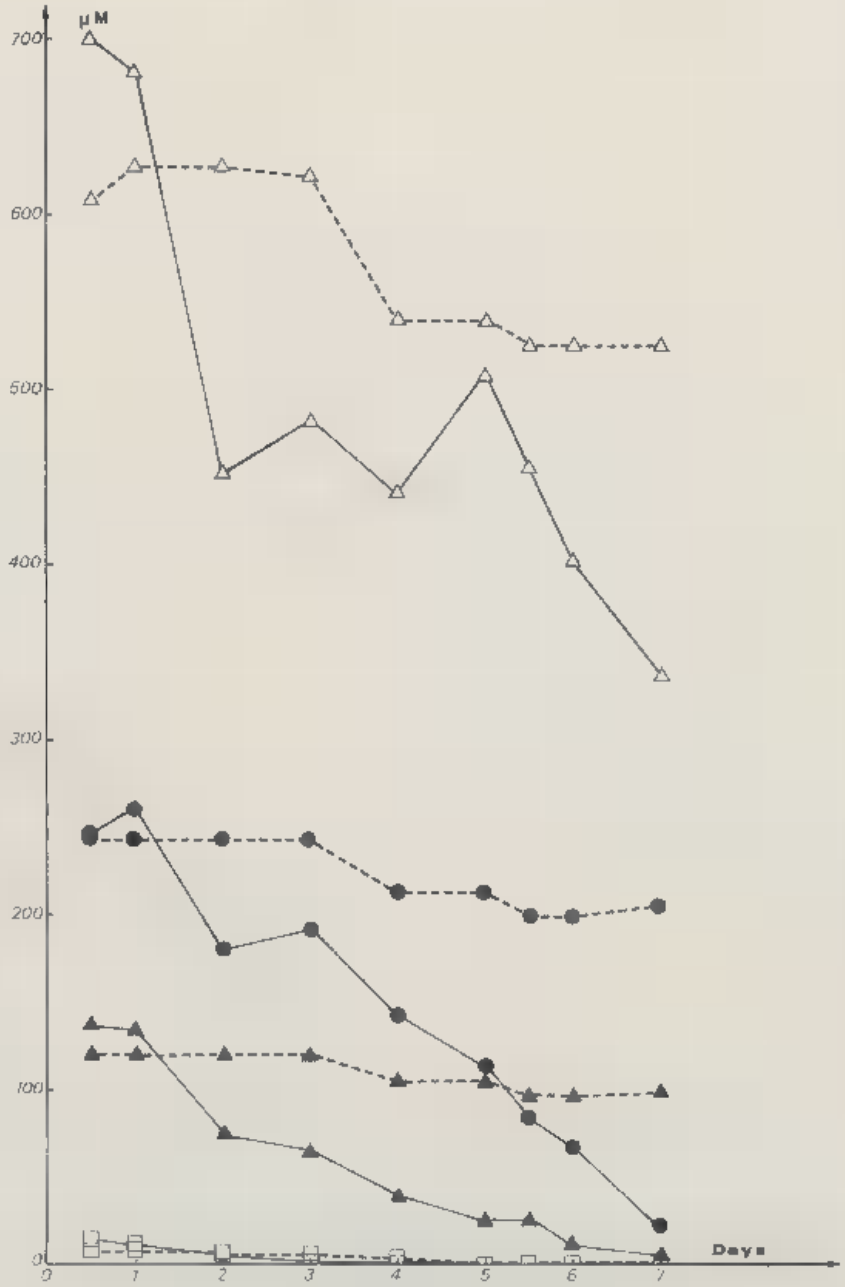

Fig. 3 — Daily water N content in the three NH_4NO_3-enriched culture tanks and in the control tank.

——— : N-NH_4, - - - - - : N-NO_3.

▲ : 0.26 mM total N, ● : 0.50 mM total N, △ : 1.26 mM total N, □ : control.

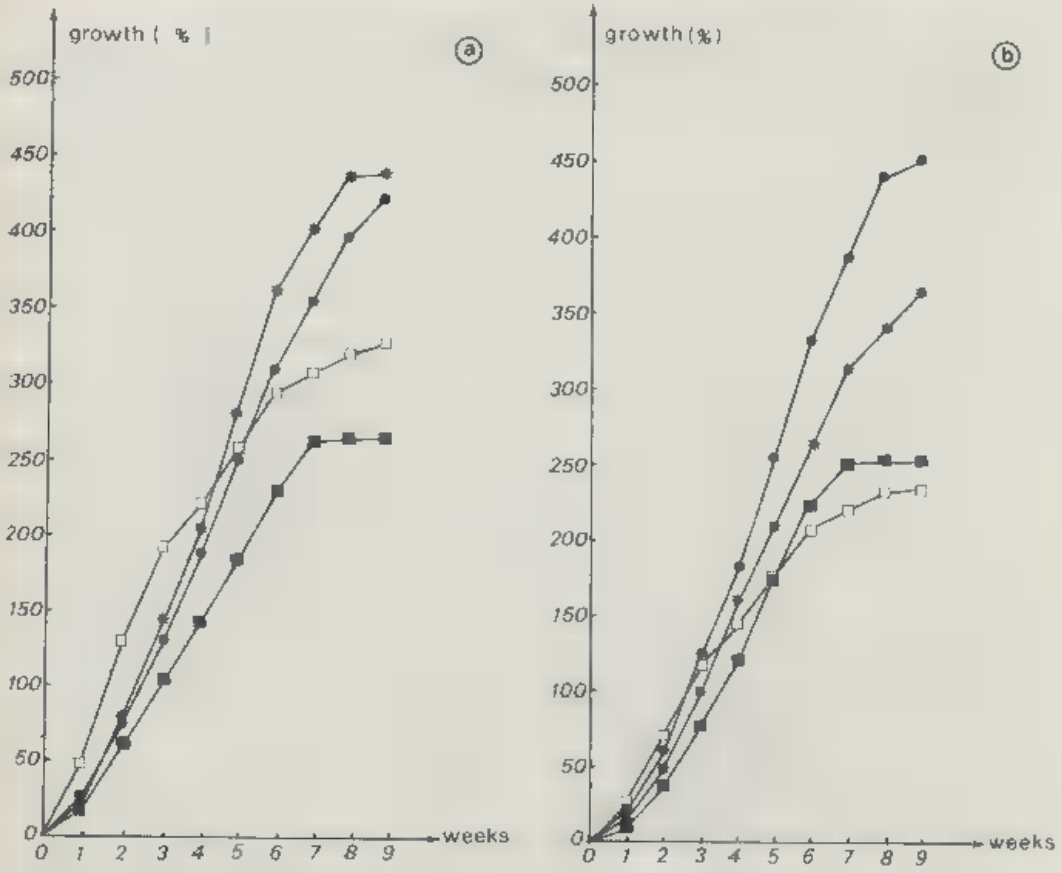

Fig. 4 — Cumulative growth of cuttings fertilized with ammonium and nitrate (NH_4NO_3) or not (controls). Results expressed in different percentages of the initial fresh weight. ● : 0.26 mM total N, ★ : 0.50 mM total N, ■ : 1.26 mM total N, □ : control.

Phosphate enrichment

As for nitrate salts, the range in phosphate supplementation values is consistently high. In such an enriched seawater, growth is poor, and the plants loose their pigmentation, with bleaching of the tips of the branches (Figs. 5d and 6d). Fragments cultivated in KH_2PO_4 and NaH_2PO_4 enriched seawater are as poorly pigmented as the control cultivated in an unenriched seawater (Table III). Values obtained for these samples are significantly lower than those resulting from material just collected. Necrosis also appears at the tips of the branches, during the last weeks of the experimentation.

Plants cultivated in a KH_2PO_4 (1) enriched medium grow significantly slower than the control plants (Table II, Fig. 7a, b). The lowest growth rate

(1) A breakdown of the feed-pump number 10 ($KH_2PO_4 = 3.7.10^{-3}$ mM) during the third week of the experimentation, resulted in the death of the cuttings cultivated in this tank. No statistical comparison can be made for this concentration.

Nature of the enrichment	Concentration of N or P (mM) added.	Chlorophyll a $\mu g.mg^{-1}$ DM	Chlorophyll c $\mu g.mg^{-1}$ DM	Total chlorophyll $\mu g.mg^{-1}$ DM	Carotenoids $\mu g.mg^{-1}$ DM
Cultivation control		0,119	0,237	0,356	$88.8.10^{-3}$
Collection control		1,400	0,301	1,701	$417.4.10^{-3}$
KNO_3	0,69	0,923	0,954	1,877	$472.8.10^{-3}$
	0,99	1,678	0,210	1,898	$409.6.10^{-3}$
$NaNO_3$	0,59	0,518	0,798	1,316	$410.4.10^{-3}$
	1,18	0,533	0,809	1,342	$414.4.10^{-3}$
	2,35	0,520	0,799	1,309	$391.2.10^{-3}$
NH_4NO_3	0,26	0,431	0,634	1,065	$339.2.10^{-3}$
	0,50	0,400	0,605	1,005	$358.4.10^{-3}$
KH_2PO_4	$14,7.10^{-3}$	0,119	0,222	0,341	$97.6.10^{-3}$
	$73,5.10^{-3}$	0,148	0,288	0,436	$116.8.10^{-3}$
NaH_2PO_4	$41,7.10^{-3}$	0,175	0,361	0,536	$125.6.10^{-3}$
	$83,3.10^{-3}$	0,108	0,184	0,292	$88.0.10^{-3}$
	$125,0.10^{-3}$	0,083	0,174	0,257	$80.0.10^{-3}$

Table III : Pigment contents of the cuttings grown at three concentrations of five different enrichments, grown in an unenriched seawater and just gathered at the collection station. DM : dry matter.

(152.2 and 136.1 % for apical and subapical segments respectively) is observed when KH_2PO_4 concentration is $73.5.10^{-3}$ mM. Cuttings cultivated in such conditions cannot survive during the experiment. The decay of the plants is observed after 7 weeks culture.

No significant difference exists between plants cultivated in a NaH_2PO_4 enriched medium and plants cultivated in unenriched seawater (Table II, fig. 7c, d). The concentrations tested seem to be too high to provide a significant enhancement of the cuttings growth, but they are not high enough to inhibit the growth.

DISCUSSION

Nitrate supplementation is found to increase growth rates. This effect is particularly important when nitrate supply does not exceed 1 mM. This influence is effective only when N added is NO_3 alone. Such a correlation between

Fig. 5 — Morphological aspects of the apical cuttings at the end of the culture.
a : Control plant, b : Apical cutting submitted to a KNO_3 (0.69 mM N-NO_3) enrichment, c : Apical cutting submitted to ■ NH_4NO_3 (0.50 mM total N) enrichment, d : Apical cutting submitted to a KH_2PO_4 ($14.7.10^{-3}$ mM P-PO_4) enrichment.

Fig. 6 — Morphological aspects of the subapical cuttings at the end of the culture.
a : Control plant, b : Subapical cutting submitted to a KNO_3 (0.69 mM N-NO_3) enrichment, c : Subapical cutting submitted to a NH_4NO_3 (0.50 mM total N enrichment), d : Subapical cutting submitted to a KH_2PO_4 ($14.7.10^{-3}$ mM P-PO_4) enrichment.

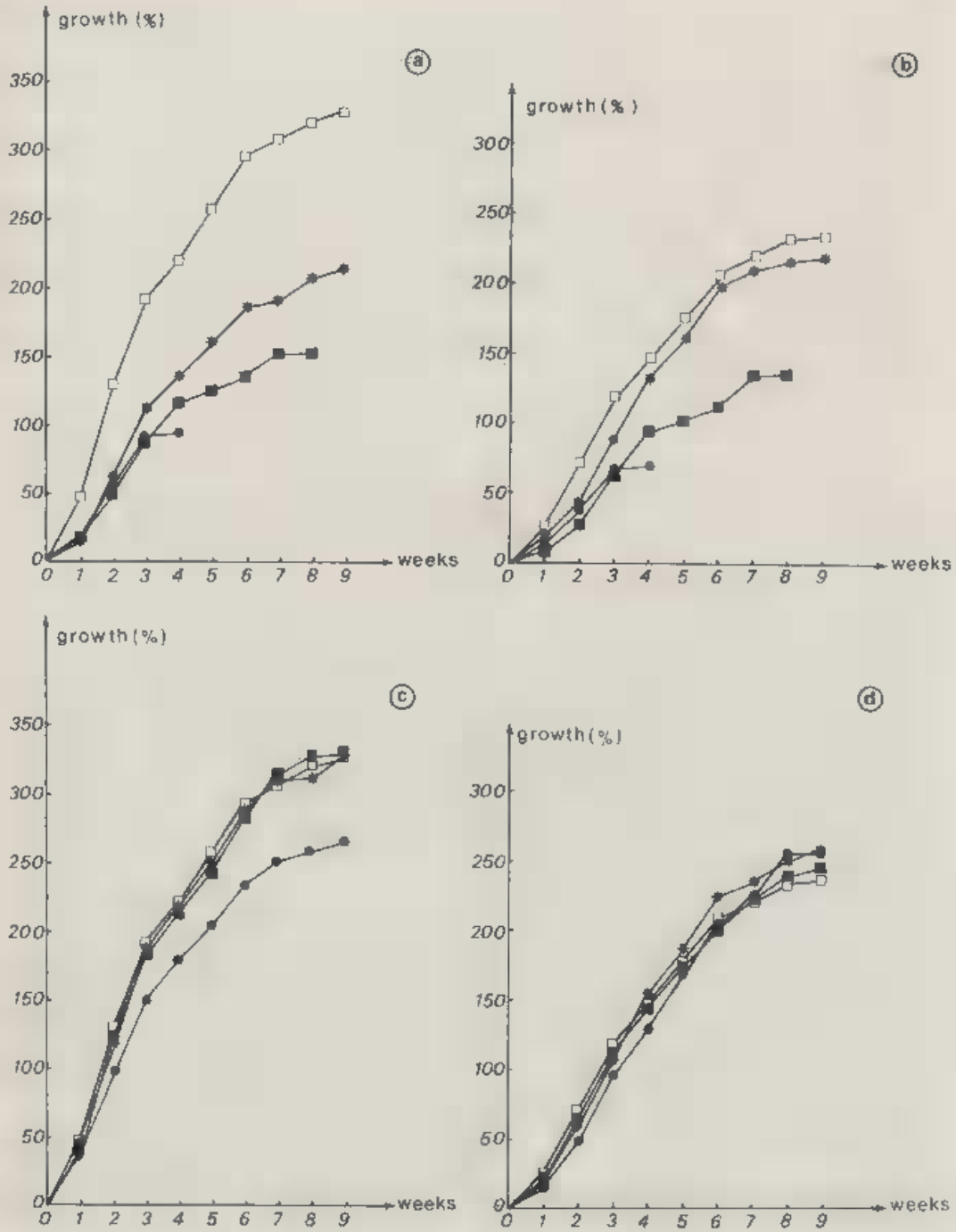

Fig. 7 — Cumulative growth of cuttings fertilized with phosphate or not (controls). Results expressed in different percentages of the initial fresh weight.

a and b : KH_2PO_4 enrichment. a : apical segments, b : subapical segments.
● : $3.7.10^{-3}$ mM P-PO_4, ★ : $14.7.10^{-3}$ mM P-PO_4, ■ : $73.5.10^{-3}$ mM P-PO_4, □ : control.

c and ▮ : NaH_2PO_4 enrichment. c : apical segments, d : subapical segments.
● : $41.7.10^{-3}$ mM P-PO_4, ★ : $83.3.10^{-3}$ mM P-PO_4, ■ : $125.0.10^{-3}$ mM P-PO_4, □ : control.

NO_3 concentration and growth has been demonstrated for other Phaeophyta in particular Laminariales. The marked seasonal variations in growth rates in the genus *Laminaria* has often been correlated to seasonal fluctuations in dissolved inorganic nutrients (essentially nitrates). Chapman & Craigie (1977), and Chapman & Lindley (1980) related growth rates of *Laminaria longicruris* and *Laminaria solidungula*, *in situ*, and inorganic nutrient changes occurring in the seawater. For *Macrocystis pyrifera*, Gerard (1982) showed that a minimum NO_3 concentration of 1 to 2 μM is necessary for the growth of this seaweed. Such a relation also exists when the seaweeds are cultivated in greenhouse tanks. The growth rate of *Laminaria saccharina* sporophytes is dependent on inorganic nitrogen in the culture medium (Chapman *et al.*, 1978; Wheeler & Weidner, 1983). There is a close relationship between growth and nutrient concentration, up to ■ saturating substrate concentration situated between 5 and 10 μM nitrate.

Nitrogen in our control tank remains at levels averaging 0.25 μM NO_3 and 0.15 μM NH_4. These values are very low and perhaps not sufficient to support a high growth rate. Pigment content is affected by these nitrogen levels. Total chlorophylls and carotenoids are 5 times higher in fresh material than in fragments cultivated in a medium with no additional nutrient supply. It is clear that with such a bleaching, *Cystoseira stricta* cuttings can not support a great development.

External concentrations of NH_4, NO_3 and NO_2 influence the uptake of these available forms of nitrogen. The uptake of NO_3 generally follows a hyperbolic curve resembling those described by the Michaelis-Menten equation (Topinka, 1978 : *Fucus spiralis*; Harlin, 1978 : *Enteromorpha* spp.; D'Elia & De Boer, 1978 : *Neogardhiella baileyi* and *Gracilaria foliifera*; Haines & Wheeler, 1978 : *Hypnea musciformis* and *Macrocystis pyrifera*). Such an uptake curve in NO_3 can result in a corresponding growth rare curve (De Boer *et al.*, 1978 : *Gracilaria foliifera* and *Neoagardhiella baileyi*).

Our data indicate that nitrate, as the sole source of nitrogen addition, enrichment results in growth rate enhancement. Similar responses has been described for many other species. Although values are higher than those determined for the genus *Laminaria* or for *Codium fragile* (Hanisak, 1979 ■ and b), they compare well with those employed by Morgan *et al.* (1980) for *Palmaria palmata* ($NaNO_3$ 1 mM added per liter of seawater). The best growth is obtained with addition of 0.69 mM NO_3 (as KNO_3). Differences observed between $NaNO_3$ and KNO_3 treated cuttings could be explained by potassium role during absorption of photosynthesis products. Growth is slower and can be inhibited when NO_3 concentration becomes more important. Further investigations are necessary to determine whether smaller concentrations could provide better results. Smaller amounts of nitrogenous fertilizer will perhaps be more favorable to the alginic acid synthesis, as it is the case for carragheenan and agar synthesis (Dawes *et al.*, 1974; De Boer, 1978, Guist *et al.*, 1982). There is an inverse relationship between phycocolloids synthesis and plant growth.

When nitrogen supply to the culture medium consists of NH_4NO_3, growth response is different. Daily analysis of the culture medium show that uptake

of NH_4 is more important than uptake of NO_3 (Fig. 3). Faster uptake and utilization of ammonium has been shown in *Macrocystis pyrifera* (Wheeler, 1982; Wheeler & Srivastava, 1984). The difference between ammonium and nitrate uptake could be explained by the greater need for reductant and energy for assimilation of nitrate than for ammonium (Syrett, 1981). In the light, ammonium does not inhibit nitrate uptake of brown algae (Wheeler & Srivastava, 1984; Topinka, 1978; Haines & Wheeler, 1978; Harlin & Craigie, 1978), contrary to the situation observed in many phytoplankton and red algae (Collos & Slawyk, 1980; De Boer, 1981). Nitrate uptake by unicellular algae stops when NH_4 concentrations exceed a certain threshold. The NH_4 threshold generally ranges between 0.5 and 1.0 μM N but it can be higher as it is in oyster- pond algae (Robert & Maestrini, 1986; Maestrini *et al.*, 1986).

The growth of *Cystoseira stricta* cuttings receiving such an enrichment is only two thirds of that obtained with the addition of nitrate alone. Nitrate and ammonium do not seem to support similar growth rates. The same results, in different culture conditions, have been obtained by Fries (1963) for *Goniotrichum* and *Nemalion*. Some of the more obvious effects of nitrogen deprivation in microalgal cultures are an accumulation of carbon reserves, ■ reduction in the rate of photosynthesis and a loss of chlorophyll (Syrett, 1981). *Cystoseira stricta* cuttings grown on NH_4 have less chlorophyll than the collection control and than the cultures supplied with NO_3 alone. Such a loss of chlorophyll could partly explain the reduced growth rate. On the other hand, the increased growth rate of the cuttings cultivated in a KNO_3 enriched medium could be partly consequent upon high chlorophyll ratio. Further experiments will be conducted in the near future to determine whether nitrogen status could influence the rate of photosynthesis. Although, in many cases, nitrate and ammonium can provide similar growth rates (Neish & Fox, 1971; Neish & Shacklock, 1971; Prince, 1974 : *Chondrus;* Topinka & Robbins, 1976 : *Fucus*). High ammonium concentrations (0.63 mM) result in an inhibition of the growth. Inhibitory effects of high ammonium concentrations have been reported for several Chlorophyta (Andersson, 1942 : *Enteromorpha* and *Ulva*; Kautsky, 1982 : *Enteromorpha*).

Phosphate supply does not increase growth rate of *Cystoseira stricta* cuttings and sometimes results in an inhibition of the growth. However, it seems to be necessary for the complete development of microscopic stages of *Lessonia nigrescens* (Hoffmann *et al.*, 1984). The patterns of development may be modified by the interactions of nitrates and phosphates (Hsiao & Druehl, 1973 : *Laminaria saccharina*; Hoffmann & Santelices, 1982; Hoffmann *et al.*, 1984 : *Lessonia nigrescens*). Experimentation with some agricultural fertilizers, shows that the best growth rates were obtained when the fertilizer added contained at the same time, nitrogen and phosphorus (Gonzales-Rodriguez & Maestrini, 1984). It is possible that nutrient requirements may change with culture mode and conditions (light, temperature. . .) and also with the development stage of the algae. The results from these P supplementation experiments indicate that the concentrations chosen are above saturation.

Another important observation leads in the fact that the pigmentation of the cuttings varies with the nature of the nutrient supply. When the culture medium is enriched with phosphates, the new branches are as lightly pigmented as cuttings cultivated in unenriched seawater. As for numerous other macrophytes, addition of nitrogen results in richly pigmented plants. The chlorophyll content and photosynthetic capacities of *Laminaria saccharina* in culture increase with increasing external NO_3 (Chapman et al., 1978), Pigment content of our NO_3 (as the sole source of nitrogen addition) enriched cuttings is similar to fresh material one. The deep-red pigmentation of *Palmaria palmata* (Morgan *et al.*, 1980; Morgan & Simpson, 1981), *Chondrus crispus* (Neish *et al.*, 1977), *Gelidium amansii* (Yamada, 1972), *Gigartina exasperata* and *Iridea cordata* Waaland, 1977), *Hypnea* (Mshigeni, 1978), receiving N supply, shows the general occurrence of this phenomenon in red algae. Our data agree with these observations but they ▬ more restrictive : cuttings ▬ darkly colored only when nitrate represents the only N source added. When nitrate supply is coupled with ammonium, pigmentation is less intensive and the tips of the new branches are bleached. There is a relationship between pigment content and growth. The observed growth enhancement by nitrate application can be explained in terms of nitrate role in pigmentation.

The information from this study may be useful in designing feeding conditions for further *Cystoseira* pilot-scale cultivation.

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REFERENCES

ANDERSSON M., 1942 – Einige ernährungsphysiologische Versuche mit *Ulva* und *Enteromorpha*. *Kungl. Fysiogr. Sällsk. Lund, Förh.* 12 : 1-11.

ARFI R., 1984 – Gulf of Fos (France) : Main hydrological features (1976-1978). *Hydrobiologia* 118 : 187-197.

CHAPMAN A.R.O. ■ CRAIGIE J.S., 1977 – Seasonal growth in *Laminaria longicruris* : relations with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.* 40 : 197-205.

CHAPMAN A.R.O. & LINDLEY J.E., 1980 – Seasonal growth of *Laminaria solidungula* in the Canadian High Arctic in relation to high irradiance and dissolved nutrient concentrations. *Mar. Biol.* 57 : 1-5.

CHAPMAN A.R.O., MARKHAM J.W. & LÜNING K., 1978 – Effects of nitrate concentration on the growth and physiology of *Laminaria saccharina* (Phaeophyta) in culture. *J. Phycol.* 14 : 195-198.

COLLOS Y. & SLAWYK G., 1980 — Nitrogen uptake and assimilation by marine phytoplankton. *In* FALKOWSKI P.G. (Ed.), *Primary productivity of the sea.* Plenum Press, New York, pp. 195-211.

DAGNELIE P., 1973 — *Théorie et méthodes statistiques, Vol. 1.* Presses Agronomiques de Gembloux (Belgique), 378 p.

DAWES C.J., LAWRENCE J.M., CHENNY D.P. & MATHIESON A.C., 1974 — Ecological studies of Florida *Eucheuma.* 3- Seasonal variation of carrageenan, total carbohydrate, protein and lipid. *Bull. Mar. Sci.* 24 : 286-299.

DE BOER J.A., 1978 — Effects of nitrogen enrichment on growth rate and phycocolloid content in *Gracilaria folilfera* and *Neoagardhiella baileyi* (Florideophyceae). *Proc. Int. Seaweed Symp.* 9 : 263-271.

DE BOER J.A., 1981 — Nutrients. *In* LOBBAN C.S. & WYNNE M.J. (Eds.), *The biology of seaweeds.* Blackwell, London, pp. 356-392.

DE BOER J.A., GUIGLI H.J., ISRAEL T.L. & D'ELIA C.F., 1978 — Nutritional studies of two red algae. I. Growth rate as a function of nitrogen source and concentration. *J. Phycol.* 14 : 261-266.

D'ELIA C.F. & DE BOER J.A., 1978 — Nutritional studies of two red algae. II. Kinetics of ammonium and nitrate uptake. *J. Phycol.* 14 : 266-272.

EPIARD-LAHAYE M., PELLEGRINI M. & WEISS H., 1987 — Influence des rythmes émersion-immersion sur le développement des boutures de *Cystoseira stricta* (Phéophycées, Fucales) en culture. *Bot. Mar.* 30 : 259-266.

FRIES L., 1963 — On the cultivation of axenic red algae. *Physiol. Plant* 16 : 695-708.

GERARD V.A., 1982 — *In situ* rates of nitrate uptake by giant kelp, *Macrocystis pyrifera* (L.) C. Agardh : tissue differences, environmental effects, and predictions of nitrogen limited growth. *J. Exp. Mar. Biol. Ecol.* 62 : 211-224.

GONZALEZ-RODRIGUEZ E. & MAESTRINI S.Y., 1984 — The use of some agricultural fertilizers for the mass production of marine algae. *Aquaculture* 36 : 245-256.

GUIST G.G. Jr., DAWES C.J. & CASTLE J.R., 1982 — Mariculture of the red seaweed, *Hypnea musciformis. Aquaculture* 28 : 375-384.

HAINES K.C. & WHEELER P.A., 1978 — Ammonium and nitrate uptake by the marine macrophytes *Hypnea musciformis* (Rhodophyta) and *Macrocystis pyrifera* (Phaeophyta). *J. Phycol.* 14 : 319-324.

HANISAK M.D., 1979a — Growth patterns of *Codium fragile* spp. *tomentosoides* in response to temperature, salinity, and nitrogen source. *Mar. Biol.* 50 : 319-332.

HANISAK M.D., 1979b — Nitrogen limitation of *Codium fragile* spp. *tomentosoides* as determined by tissue analysis. *Mar. Biol.* 50 : 333-337.

HARLIN M.M., 1978 — Nitrate uptake by *Enteromorpha* spp. *(Chlorophyceae)* : applications to aquaculture systems. *Aquaculture* 15 : 373-376.

HARLIN M.M. & CRAIGIE J.S., 1978 — Nitrate uptake by *Laminaria longicruris* (Phaeophyceae). *J. Phycol.* 14 : 464-467.

HOFFMANN A.J. & SANTELICES B., 1982 — Effects of light intensity and nutrients on gametophytes and gametogenesis of *Lessonia nigrescens* Bory (Phaeophyta). *J. Exp. Mar. Biol. Ecol.* 60 : 77-89.

HOFFMANN A.J., AVILA M. & SANTELICES B., 1984 — Interactions of nitrate and phosphate on the development of microscopic stages of *Lessonia nigrescens* Bory (Phaeophyta). *J. Exp. Mar. Biol. Ecol.* 78 : 177-186.

HSIAO S.I.C. & DRUEHL L.D., 1973 — Environmental control of gametogenesis in *Lami-*

naria saccharina. II. Correlation of nitrate and phosphate concentrations with gametogenesis and selected metabolites. *Canad. J. Bot.* 51 : 829-840.

JEFFREY S.W. & HUMPHREY G.F., 1975 – New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* 167 : 191-194.

KAUTSKY L., 1982 – Primary production and uptake kinetics of ammonium and phosphate by *Enteromorpha compressa* in an ammonium sulfate industry outlet area. *Aquatic Botany* 12 : 23-40.

KOROLEFF F., 1969 - Direct determination of ammonia in sea water as rubazoic acid. *ICES Paper* C. M. : 1-9.

MAESTRINI S.Y., ROBERT J.M., LEFTLEY J.W. & COLLOS Y., 1986 – Ammonium thresholds for simultaneous uptake of ammonium and nitrate by oyster-pond algae. *J. Exp. Mar. Biol. Ecol.* 102 : 75-98.

MORGAN K.C. & SIMPSON F.J., 1981 – Cultivation of *Palmaria (Rhodymenia) palmata* : effect of high concentrations of nitrate and ammonium on growth and nitrogen uptake. *Aquatic Botany* 11 : 167-171.

MORGAN K.C., SHACKLOCK P.F. & SIMPSON F.J., 1980 – Some aspects of the culture of *Palmaria palmata* in greenhouse tanks. *Bot. Mar.* 23 : 765-770.

MSHIGENI K.E., 1978 – Effects of nitrate fertilizer on the growth of the economic seaweed *Hypnea* Lamouroux (Rhodophyta, Gigartinales). *Nova Hedwigia* 29 : 231-236.

MURPHY J. & RILEY J.P., 1962 – A modified single solution method for the determination of dissolved inorganic phosphates. *Anal. Chim. Acta* 14 : 318-319.

NEISH A.C. & FOX C., 1971 – Greenhouse experiments on the vegetative propagation of *Chondrus crispus* (Irish Moss). *Technical Report Series n° 12, NRCC n° 12034*, 68 p. typescript.

NEISH A.C. & SHACKLOCK P.F., 1971 – Greenhouse experiments on the propagation of strain T_4 of Irish Moss. *Atlantic Reg. Tech. Rep. Ser. n° 14, National Research Council, Canada, Halifax*, 25 p.

NEISH A.C., SHACKLOCK P.F., FOX C.H. & SIMPSON F.J., 1977 – The cultivation of *Chondrus crispus*. Factors affecting growth under greenhouse conditions. *Canad. J. Bot.* 55 : 2263-2271.

PELLEGRINI M. & LAHAYE M., 1987 – A tide-generating apparatus for laboratory cultures of marine algae. *Aquacultural Engineering* 6 : 183-189.

PRINCE J.S., 1974 – Nutrient assimilation and growth of some seaweeds in mixtures of seawater and secondary seawage treatment. *Aquaculture* 4 : 69-79.

ROBERT J.M.& MAESTRINI S.Y., 1986 – Absorptions simultanées des ions NO_3^- et NH_4^+ par trois diatomées de claires à huîtres, en culture axénique. *Phycologia* 25 : 152-159.

SYRETT P.J., 1981 – Nitrogen metabolism of microalgae. *Can. Bull. Fish. Aquatic Sci.* 210 : 182-210.

TOPINKA J.A., 1978 - Nitrogen uptake by *Fucus spiralis* (Phaeophyceae). *J. Phycol.* 14 : 241-247.

TOPINKA J.A. & ROBBINS J.V., 1976 – Effect of nitrate and ammonium enrichment on growth and nitrogen physiology in *Fucus spiralis*. *Limnol. Oceanogr.* 21 : 659-664.

WAALAND J.R., 1977 – Growth of Pacific Northwest marine algae in semi-closed culture. KRAUSS R.W. (Ed.), *The Marine Plant Biomass of the Pacific Northwest Coast*. Oregon State University Press, pp. 117-137.

WHEELER W.N., 1982 – Nitrogen nutrition of *Macrocystis*. SRIVASTAVA L.M. (Eds.),

Synthetic and degradative processes in marine macrophytes, Walter De Gruyter, Berlin, pp. 121-137.

WHEELER W.N. & SRIVASTAVA L.M., 1984 – Seasonal nitrate physiology of *Macrocystis integrifolia*. *J. Exp. Mar. Biol. Ecol.* 76 : 35-50.

WHEELER W.N. & WEIDNER M., 1983 – Effects of external inorganic nitrogen concentration on metabolism, growth and activities of key carbon and nitrogen assimilatory enzymes of *Laminaria saccharina* (Phaeophyceae) in culture. *J. Phycol.* 19 : 92-96.

YAMADA N., 1972 – Manuring for *Gelidium*. NISIZAWA (Ed.). *Proc. Int. Seaweed Symp.* 7 : 385-390.